

In vitro colorimetric evaluation of the efficacy of various bleaching methods and products

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Objective: Various bleaching modalities are now offered to patients, either monitored by the dental office or self-directed, for which relative efficacy is unknown. The aim of this in vitro study was to evaluate the ability of different bleaching products and protocols to lighten enamel and dentin. **Method and Materials:** Bovine tooth samples of standardized thickness (2.5 ± 0.025 mm with similar dentin and enamel thicknesses) were prepared and stained with whole blood and hemolysate before being submitted to 11 different bleaching regimens: home bleaching using 10%, 15%, 16%, or 20% carbamide peroxide, power bleaching using 15% hydrogen peroxide, 30% hydrogen peroxide, or 25% carbamide peroxide with or without light activation, and over-the-counter bleaching strips containing 5.3% hydrogen peroxide. Colorimetric measurements were performed on each sample side, according to the CIE L*a*b* system, before and after staining as well as after each series of 5 bleaching sessions (number of applications varied from 5 to 20 times, according to the bleaching protocol). **Results:** All products and protocols produced a similar bleaching effect on enamel, while the home bleaching regimen proved largely superior to lighten dentin. **Conclusion:** In-office bleaching techniques proved less efficient than home bleaching for removing stains deposited in dentin. (*Quintessence Int* 2006;37:515–526)

Key words: carbamide peroxide, home bleaching, hydrogen peroxide, in-office bleaching, power bleaching, tooth bleaching

The use of hydrogen peroxide to bleach teeth was first reported in 1884.¹ In 1918, Abbot described the chairside bleaching

method, as it is known today, using 35% hydrogen peroxide together with heat and light to boost the oxidation reaction. Initially, vital bleaching techniques were aimed at correcting severe discolorations, mainly endemic fluorosis.^{2–5} The technique remained confidential until its use for tetracycline-stained teeth was fostered by the increased use of these drugs.⁶ Later, bleaching also became a cosmetic procedure, which significantly expanded its use.⁷ Bleaching became even more popular after it was proposed for at-home application.⁸ Since then, millions of patients have satisfactorily bleached their teeth using the home bleaching technique, an inexpensive yet simple procedure compared to traditional chairside methods.⁸

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With the exception of a few other products such as oxalic acid, chlorine, and muriatic acid, hydrogen peroxide (H_2O_2) was used as the main active ingredient for all kinds of bleaching techniques.¹ Carbamide peroxide (CP), used formerly for topical disinfection following oral surgery, was found to be an interesting alternative source of H_2O_2 , providing a slower release of active, oxidizing ions.⁸ Today, bleaching products are frequently found in the form of gels containing varying concentrations of carbamide peroxide or H_2O_2 , depending on the application methods, combined with a few other substances like stabilizers, catalysts, and desensitizing and flavor agents. Lower concentrations of both carbamide peroxide and H_2O_2 are used for home bleaching, while higher concentrations are necessary for in-office treatments. Chairside techniques also make use of intense light-sources (halogen, laser, plasma-activated curing, light-emitting diode [LED]), or chemicals to activate and accelerate the degradation of the bleaching gel; this approach is known as “power bleaching.”⁹

Despite manufacturers’ claims and some optimistic marketing statements, it is not known whether all bleaching products and techniques are equally effective. Actually, because diffusion within the dental tissues is mostly governed by concentration and the application time of the active substances,¹⁰ the efficacy of the different protocols and product concentrations is likely to vary. Clinical reports demonstrated the clinical efficacy and safety of home bleaching techniques,^{11–14} while there is only scarce information about the potency of chairside application methods. The few studies available suggest that the effect produced by a single chairside application of H_2O_2 gel is almost inconspicuous, making questionable the real value of many “power bleaching” systems.^{15,16} The limitation of chairside techniques might be related to the limited application time, which might not be fully compensated by the use of higher concentrations of active ingredients. Moreover, relatively long clinical experience gained with former chairside bleaching techniques (making use of the same bleaching product and activation principle) has also shown the limits of this approach.¹⁷

It is also quite difficult to compare the effect of the numerous products and application protocols because there is no consensus about how the efficacy of bleaching techniques should be assessed. In clinical trials, patients’ self-appreciation, intraoral photographs, comparisons to shade guides, and spectrophotometric/colorimetric measurements have so far been used.^{18–20} In addition to the numerous evaluation methods employed, there is a large variation in the post-treatment observation-measurement intervals. Moreover, one has to take into account the limitations of in vivo trials, which naturally vary from clinical conditions, as well as the limited number of products, variables, and parameters that can be simultaneously assessed. While numerous in vitro studies were designed to assess the effect of bleaching techniques on tooth composition and microstructure,^{21–26} little was undertaken to measure their effects on tooth color or specific lightening potential. Staining techniques were developed to assess the lightening effect of different bleaching techniques but mainly for nonvital teeth.^{27–29} This approach, however, presents an interesting potential for evaluating vital bleaching techniques as well.^{30,31}

Because of the tremendous interest of patients for cosmetic dental procedures and bleaching in particular, the industry has tried to broaden the choice of application methods, with modifications or improvements of classic home and chairside techniques. Today, clinicians and patients are presented with a large selection of bleaching systems but know very little about the relative effect of these many options. The aim of the present study, then, was to test the hypothesis that different bleaching products and protocols have the potential to lighten the color of enamel and dentin fragments of discolored bovine teeth at various degrees. For this purpose, a standardized staining technique of the samples was used, and all color changes were assessed in vitro with a colorimeter.

METHOD AND MATERIALS

Preparation and staining of samples

Fifty-five permanent bovine maxillary incisors, collected from animals sacrificed at the age of 18 months (± 1 month), were used for this study. After careful cleaning with pumice, the buccal surface of each tooth was flattened with a model trimmer to obtain an even surface of 14×8 mm (± 1 mm). Roots were cut about 1 mm below the cemento-enamel junction. Each tooth was then embedded in a self-curing acrylic resin (Technovit 4071, Heraeus-Kulzer), the flattened area facing the mold base. Embedded samples were trimmed again to a thickness of about 2.6 mm and then were further polished on both sides with sandpaper of decreasing grits (250, 500, and 2,400 μm) (LaboPol-II, Struers), providing a finished section 2.5 mm (± 0.05 mm) thick (about half enamel and half dentin in thickness) with aforementioned width and length dimensions (Fig 1).

Colorimetric measurements (initial measurements, denoted as I) of samples were performed on each side (dentin and enamel) using a reflectance colorimetric device (Minolta CR-21, Minolta). This device was set to produce color parameters based on average daylight (D65: 6504 K). The optical geometry of this system consists of a 45-degree illumination angle and a 0-degree (normal) observation angle. The color parameters were recorded in the $L^*a^*b^*$ color space, as established by the Commission Internationale de l'Éclairage (CIE) in 1976.³²

Samples were stained with human blood, using the technique described by Freccia and Peters,²⁷ with slight modifications related to the use of tooth fragments rather than whole tooth crowns. Enamel and dentin surfaces were first etched with a 35% phosphoric acid gel (Ultraetch, Ultradent) for 30 and 15 seconds, respectively, to remove smear layers. Samples (5 per group) were then randomly immersed in test tubes ($n = 11$) containing whole blood and centrifuged at 4,000 rpm for 30 minutes, 3 times a day for 3 consecutive days. At completion of this first step, only the hemolysate was recuperated for further hemolysis of the red blood cells. The hemolysate was mixed with an

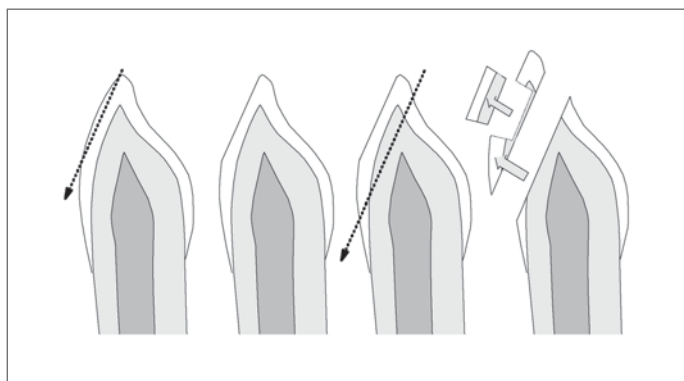


Fig 1 Preparation of bovine tooth sections. After the section plane was defined, a cut was made 2.5 mm (± 0.05 mm) below the surface. A fragment with 14×8 mm (± 1 mm) dimensions, comprising both dentin and enamel tissues, was removed from the section.

equal amount of distilled water and centrifuged twice at 4,000 rpm for 30 minutes. Thereafter, the samples were immersed again in the new hemolysate and centrifuged at 4,000 rpm for 30 minutes, 3 times a day, for 3 consecutive days. Samples were stored in a humid environment for 7 days before proceeding with new $L^*a^*b^*$ colorimetric measurements on each sample side (post-coloration measurements, denoted as PC).

Bleaching procedures

Table 1 describes the composition of all bleaching products under evaluation and their application protocol. Bleaching products were applied as a 1-mm-thick layer, on enamel surfaces only. All procedures/products were tested in 3 consecutive phases: 2 phases of 5 applications and 1 phase of 10 applications (total of 20 applications). Samples treated with Opalescence Xtra Boost and BriteSmile (chairside bleaching) received only 10 and 5 applications, respectively, of the bleaching product. The number of applications closely followed the manufacturer's instructions for each bleaching protocol.

$L^*a^*b^*$ colorimetric measurements of both sample sides were performed after each test phase (posttreatment measurements, denoted as A, B, and C).

Table 1 Bleaching products under evaluation

Product (manufacturer)	Active ingredient	Bleaching method	Application method	Batch number
O10 Opalescence 10% (Ultradent)	Carbamide peroxide 10%	Home bleaching	20 × 10 h	—
O15 Opalescence 15% (Ultradent)	Carbamide peroxide 15%	Home bleaching	20 × 10 h	—
O20 Opalescence 20% (Ultradent)	Carbamide peroxide 20%	Home bleaching	20 × 10 h	—
OQ Opalescence Quick (Ultradent)	Carbamide peroxide 35%	Chairside bleaching	10 × 15 min	7084MND
OXB Opalescence X-tra Boost (Ultradent)	Hydrogen peroxide 30%	Chairside bleaching with chemical activation	10 × 15 min	387MF5
N10 Nite White Excel2 10% (Discus Dental)	Carbamide peroxide 10%	Home bleaching	20 × 10 h	01337022/1127
N16 Nite White Excel2 16% (Discus Dental)	Carbamide peroxide 16%	Home bleaching	20 × 10 h	012889003/1114
D75 Day White2 (Discus Dental)	Hydrogen peroxide 7.5%	Home bleaching	20 × 2 × 30 min	01220011
WS White Strips (Procter & Gamble)	Hydrogen peroxide 5.3%	Self-directed	20 × 10 h	1269BTC1C
NL10 Nite White Excel2 “thin layer” (Discus Dental)	Carbamide peroxide 10%	Home bleaching	20 × 10 h	01337022/1127
PB Brite Smile (Discus Dental)	Hydrogen peroxide 15%	Power chairside bleaching with light activation (LED)	5 × 20 min	—

Comparison of colorimetric measurements

Color differences for each sample were calculated between the initial measurement and the postcoloration situation and between the initial measurement and each series of bleaching gel application, using the following equation^{32,33}:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

Statistics

All results for both dentin and enamel sides were submitted to a parametric statistical analysis.³⁴ Differences in L*a*b* values before and after coloration were tested with a repeated-measures analysis of variance (ANOVA) and Sheffe F test. Comparison of L*a*b* values for the 11 bleaching products and protocols was also tested with a repeated-measures ANOVA and Sheffe F test. Differences in L*a*b* values following subsequent applications of bleaching products was tested with a factorial ANOVA and Sheffe F test. All tests were carried out at a 5% level of significance.

RESULTS

L*a*b* values for both enamel and dentin sides, before and after coloration as well as after each treatment phase, are presented in Tables 2a and 2b. Color differences for both enamel and dentin sides, which correspond to ΔE between initial L*a*b* values and postcoloration as well as the 3 successive treatment phases, are presented in Table 3. Figures 2 and 3 depict color changes following staining and all subsequent bleaching procedures for both enamel and dentin sides.

Color difference between pre- and postcoloration (ΔE1) for enamel and dentin varied from 19.3 to 29.5 and 33.8 to 46.6, respectively, with no significant difference among groups. A few significant differences were found only as regards the dentin postcoloration a* values, due to slight variations in the oxidation degree of hemoglobin before treatment onset. The staining of samples was also characterized by a clear drop in L* enamel and dentin values and an increase in a* values (see Tables 2a and 2b and Figs 2a, 2b, 3a, and 3b).



Table 2a Colorimetric measurements of enamel (L*a*b* ± SD), after the different experiment phases

Product*	Initial			Postcoloration			+5 applications			+5 applications			+10 applications		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
O10	70.1 (1.3)	-0.9 (0.6)	3.3 (0.4)	44.4 (4.3)	-0.5 (1.1)	8.0 (4.0)	68.9 (5.0)	-1.8 (0.5)	-3.1 (3.3)	71.2 (4.8)	-1.2 (0.3)	-1.9 (2.5)	71.6 (3.6)	-0.9 (0.5)	0.1 (3.1)
O15	69.2 (1.3)	-1.2 (0.3)	4.7 (1.6)	45.3 (2.5)	2.9 (1.3)	5.0 (4.0)	68.6 (2.7)	-2.2 (0.3)	-2.3 (1.6)	73.3 (4.4)	-1.2 (0.4)	-0.2 (1.9)	74.0 (4.7)	-1.2 (0.4)	2.6 (3.8)
O20	68.7 (1.1)	-1.3 (0.5)	3.4 (1.9)	50.3 (7.2)	-0.7 (1.9)	2.4 (6.7)	69.3 (8.6)	-1.6 (0.7)	-4.2 (5.0)	71.2 (6.2)	-1.3 (0.7)	-2.1 (4.7)	74.5 (7.7)	-1.2 (0.6)	1.0 (5.2)
OQ	70.9 (2.5)	-1.3 (0.2)	1.7 (2.0)	46.3 (3.9)	2.0 (2.8)	9.3 (1.8)	65.2 (3.1)	-1.6 (0.2)	-6.8 (2.4)	69.2 (6.6)	-1.5 (0.4)	-4.8 (3.6)	70.0 (5.0)	-1.4 (0.2)	-1.3 (4.2)
OXB	70.7 (0.9)	-1.0 (0.3)	3.8 (2.7)	44.2 (5.9)	2.2 (1.9)	8.7 (3.4)	68.3 (3.1)	-1.0 (0.4)	-3.9 (3.7)	62.9 (3.2)	-0.9 (0.4)	-3.4 (4.5)	—	—	—
N10	68.9 (1.5)	-1.7 (0.5)	6.0 (2.6)	41.6 (6.4)	0.5 (1.4)	4.2 (3.9)	71.3 (4.6)	-1.9 (0.6)	9.1 (4.5)	74.4 (2.6)	-2.2 (0.3)	6.3 (2.4)	78.3 (3.2)	-2.1 (0.5)	5.2 (2.3)
N16	71.0 (1.5)	-1.4 (0.3)	4.2 (1.1)	42.1 (6.6)	1.5 (2.6)	8.1 (2.0)	75.7 (4.0)	-2.3 (0.4)	6.3 (2.1)	78.4 (2.6)	-2.0 (0.4)	4.8 (1.3)	81.2 (1.1)	-2.1 (0.2)	5.1 (1.3)
D75	70.1 (1.6)	-1.7 (0.3)	4.2 (0.7)	42.5 (3.8)	-0.5 (1.2)	3.6 (0.9)	71.1 (6.4)	-2.3 (0.3)	4.9 (3.2)	72.7 (2.6)	-2.3 (0.4)	5.2 (2.6)	80.0 (2.5)	-2.3 (0.2)	5.1 (3.0)
WS	68.8 (2.3)	-1.3 (0.5)	1.7 (1.4)	49.4 (3.3)	1.8 (3.3)	5.6 (2.3)	63.5 (4.5)	-0.3 (0.6)	-4.2 (2.3)	67.5 (5.5)	-0.7 (0.7)	-4.4 (1.2)	70.5 (7.2)	-0.4 (0.5)	-4.7 (1.8)
NL10	70.3 (2.7)	-1.1 (0.3)	1.4 (1.1)	50.5 (4.6)	1.6 (1.7)	3.5 (2.8)	57.3 (6.5)	-1.7 (0.6)	-6.6 (4.8)	59.7 (6.4)	-1.1 (0.6)	-6.8 (4.5)	62.4 (5.7)	-0.7 (0.7)	-6.4 (2.7)
PB	69.4 (1.3)	-1.1 (0.7)	1.6 (1.0)	47.5 (5.4)	1.3 (1.5)	5.5 (1.3)	60.2 (2.9)	-0.3 (0.3)	-7.9 (2.8)	—	—	—	—	—	—

*See Table 1 for product abbreviations.

Table 2b Colorimetric measurements of dentin (L*a*b* ± SD), after the different experiment phases

Product*	Initial			Postcoloration			+5 applications			+5 applications			+10 applications		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
O10	70.3 (1.3)	-0.5 (0.7)	7.1 (1.8)	29.3 (2.5)	6.5 (2.7)	6.9 (2.1)	34.4 (4.1)	2.6 (1.9)	11.5 (4.7)	40.0 (11.9)	1.9 (1.6)	13.8 (5.0)	53.5 (10.4)	2.1 (1.0)	14.9 (4.8)
O15	69.8 (2.0)	-0.8 (0.5)	6.9 (2.0)	26.3 (5.2)	1.8 (1.1)	6.5 (3.1)	28.9 (4.5)	2.4 (1.1)	7.1 (1.4)	35.9 (9.8)	2.5 (1.9)	10.4 (3.0)	54.7 (16.6)	2.0 (2.1)	12.6 (1.6)
O20	68.3 (3.7)	-0.7 (0.7)	6.6 (1.3)	27.9 (5.0)	5.3 (1.4)	5.3 (1.9)	34.3 (5.4)	3.3 (1.7)	9.9 (5.2)	44.2 (10.8)	1.5 (2.3)	13.3 (7.0)	53.6 (18.7)	2.5 (2.1)	16.0 (4.2)
OQ	72.3 (2.3)	-0.8 (0.2)	5.9 (1.0)	23.4 (3.1)	2.2 (1.4)	10.4 (6.2)	32.4 (6.4)	3.3 (2.2)	10.4 (3.9)	34.1 (9.2)	3.1 (2.1)	10.0 (4.7)	45.5 (13.2)	2.2 (2.4)	14.0 (2.1)
OXB	69.0 (2.4)	-0.8 (0.3)	6.9 (1.9)	26.3 (5.4)	3.9 (0.8)	4.9 (2.6)	31.9 (5.5)	2.5 (1.0)	10.7 (3.0)	31.3 (8.5)	1.5 (1.2)	10.0 (5.7)	—	—	—
N10	68.9 (3.2)	-1.4 (0.7)	8.3 (2.6)	32.0 (3.7)	3.0 (1.3)	9.4 (2.3)	67.1 (4.5)	-3.3 (1.0)	17.1 (3.7)	72.9 (2.6)	-2.8 (0.5)	13.3 (1.3)	78.2 (3.5)	-2.6 (0.5)	9.7 (1.1)
N16	72.0 (2.8)	-1.2 (0.5)	5.6 (2.1)	30.1 (5.4)	4.1 (1.0)	9.4 (1.9)	63.2 (8.6)	-2.4 (0.8)	20.0 (6.7)	75.3 (1.5)	-3.1 (0.5)	15.3 (3.2)	81.7 (0.9)	-2.4 (0.4)	8.0 (2.6)
D75	67.2 (2.4)	-2.0 (0.3)	7.5 (2.0)	33.8 (6.1)	1.7 (1.1)	9.0 (2.2)	65.9 (9.3)	-3.0 (0.8)	15.7 (1.2)	76.3 (2.5)	-2.7 (0.4)	9.0 (1.2)	80.8 (2.4)	-2.2 (0.2)	6.4 (2.1)
WS	68.6 (4.5)	-0.6 (0.6)	5.7 (2.0)	24.6 (1.7)	5.6 (1.4)	2.5 (1.4)	36.2 (5.5)	3.2 (0.6)	11.3 (2.2)	41.2 (6.5)	2.9 (0.9)	13.5 (1.4)	35.3 (3.3)	3.9 (1.9)	11.9 (2.0)
NL10	66.6 (2.1)	-0.6 (1.1)	8.1 (3.0)	25.8 (4.0)	5.8 (2.0)	4.1 (2.7)	25.7 (0.9)	4.0 (0.8)	3.9 (1.1)	29.0 (1.6)	4.9 (1.3)	5.6 (3.3)	27.9 (2.6)	4.9 (0.8)	5.7 (3.4)
PB	69.2 (0.9)	-0.2 (0.8)	6.4 (1.5)	25.1 (2.7)	6.6 (0.7)	3.7 (0.8)	38.2 (7.5)	1.5 (0.8)	7.9 (2.7)	—	—	—	—	—	—

*See Table 1 for product abbreviations.



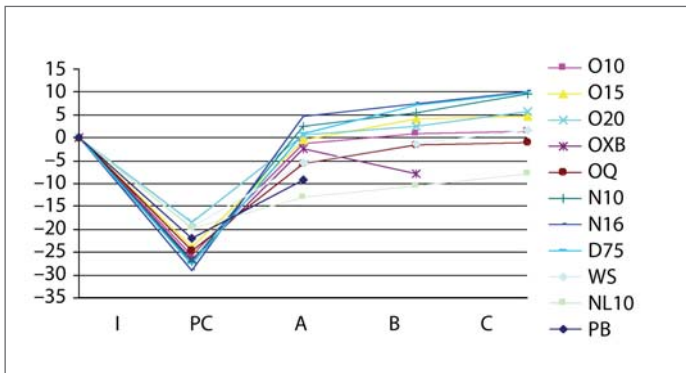


Fig 2a Differential L* enamel values following sample staining and subsequent applications of bleaching products (I = initial, PC = postcoloration, A = first 5 applications, B = total of 10 applications, and C = total of 20 applications; see Table 1 for product abbreviations).

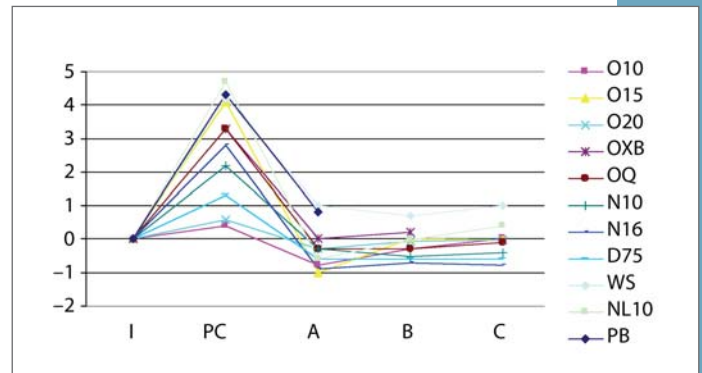


Fig 2b Differential a* enamel values following sample staining and subsequent applications of bleaching products (I = initial, PC = postcoloration, A = first 5 applications, B = total of 10 applications, and C = total of 20 applications; see Table 1 for product abbreviations).

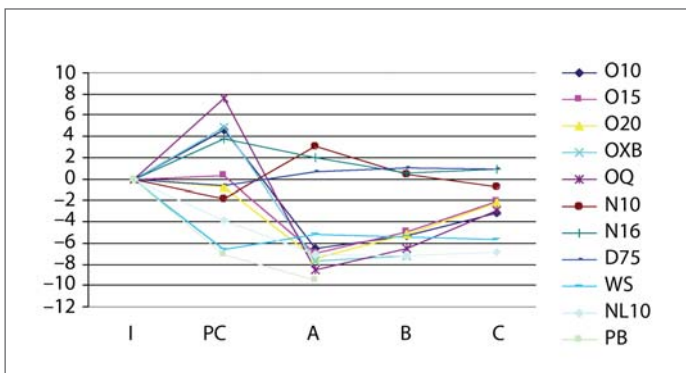


Fig 2c Differential b* enamel values following sample staining and subsequent applications of bleaching products (I = initial, PC = postcoloration, A = first 5 applications, B = total of 10 applications, and C = total of 20 applications; see Table 1 for product abbreviations).

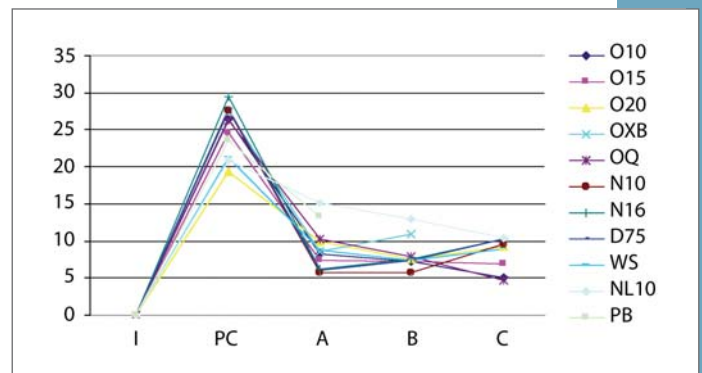


Fig 2d Global enamel color changes ΔE following sample staining and subsequent applications of bleaching products (I = initial, PC = postcoloration, A = first 5 applications, B = total of 10 applications, and C = total of 20 applications; see Table 1 for product abbreviations).

Table 3 ΔE values (SD) for dentin and enamel

Product*	Enamel				Dentin			
	ΔE1	ΔE2	ΔE3	ΔE4	ΔE1	ΔE2	ΔE3	ΔE4
O10	26.5 (4.5)	8.2 (2.6)	7.2 (1.3)	5.0 (1.7)	41.6 (2.2)	36.6 (4.0) ^{a,e}	32.4 (9.1) ^a	20.4 (7.6) ^{a,c}
O15	24.5 (3.5)	7.4 (0.8)	7.3 (1.0)	6.9 (2.1)	43.7 (5.9)	41.1 (6.1) ^a	34.5 (10.5) ^a	18.9 (3.3) ^{a,c}
O20	19.3 (6.8)	10.0 (4.5)	7.6 (3.1)	9.2 (3.1)	41.0 (6.1)	34.8 (7.2) ^{a,e}	27.4 (6.2) ^{a,c}	22.4 (15.0) ^{a,c}
OQ	26.2 (4.3)	10.3 (1.9)	7.9 (2.6)	4.7 (3.8)	46.6 (6.2)	40.6 (6.6) ^a	38.9 (9.0) ^a	28.7 (10.7) ^{a,c}
OXB	27.5 (6.3)	8.5 (2.5)	10.9 (3.0)	—	46.0 (3.7)	37.7 (5.5) ^a	38.6 (6.2) ^a	—
N10	27.6 (6.4)	5.8 (3.0)	5.7 (2.9)	9.6 (3.2)	37.2 (6.5)	9.9 (5.7) ^{b,d}	7.4 (1.9) ^b	9.8 (1.4) ^a
N16	29.5 (6.5)	6.3 (2.2)	7.6 (2.6)	10.3 (2.0)	42.5 (6.2)	19.3 (7.4) ^{c,e}	11.4 (2.6) ^{b,c,e}	10.5 (2.3) ^a
D75	27.7 (5.3)	6.0 (4.0)	7.4 (2.4)	10.2 (1.6)	33.8 (7.7)	11.6 (5.6) ^b	9.6 (3.3) ^{b,c}	14.1 (1.5) ^{a,d}
WS	21.2 (5.1)	8.7 (3.6)	7.4 (2.1)	9.0 (2.7)	44.6 (3.2)	33.1 (9.6) ^d	28.8 (9.9) ^{d,e}	34.2 (7.7) ^{b,c}
NL10	20.9 (6.1)	15.1 (6.6)	12.9 (6.7)	10.5 (5.3)	41.7 (5.3)	41.6 (0.9) ^d	38.4 (2.2) ^d	39.5 (2.2) ^{b,c,d}
PB	23.6 (6.0)	13.3 (2.7)	—	—	44.7 (3.3)	31.1 (8.2) ^d	—	—

*See Table 1 for product abbreviations.

Color differences relative to untreated samples: ΔE1 = postcoloration; ΔE2 = after the first 5 applications of bleaching agent; ΔE3 = after 5 more applications of bleaching agent (total of 10 applications); ΔE4 = after 10 more applications of bleaching agent (total of 20 applications). Groups with same lowercase letter are not statistically different (Fisher's test). Columns without letter showed no statistical difference between groups.

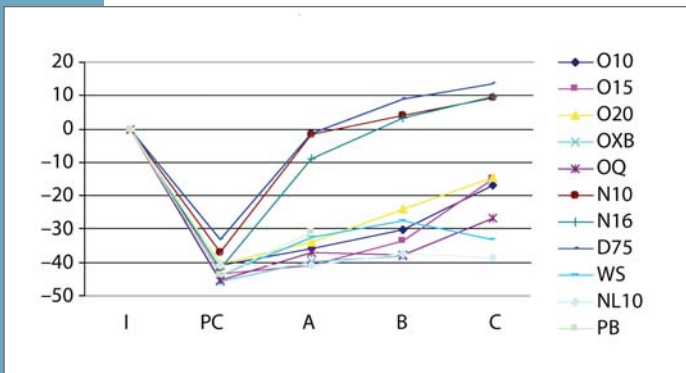


Fig 3a Differential L* dentin values following sample staining and subsequent applications of bleaching products (I = initial, PC = postcoloration, A = first 5 applications, B = total of 10 applications, and C = total of 20 applications; see Table 1 for product abbreviations).

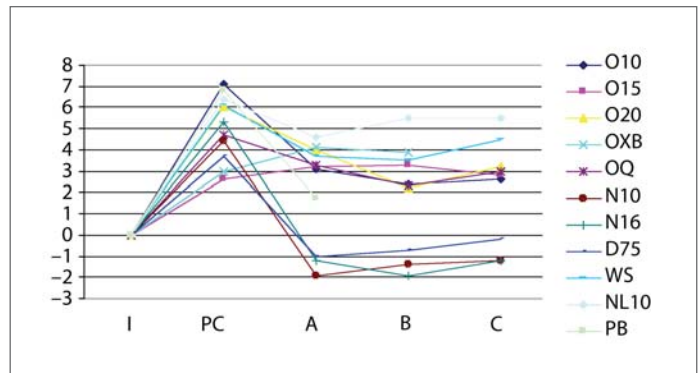


Fig 3b Differential a* enamel values following sample staining and subsequent applications of bleaching products (I = initial, PC = postcoloration, A = first 5 applications, B = total of 10 applications, and C = total of 20 applications; see Table 1 for product abbreviations).

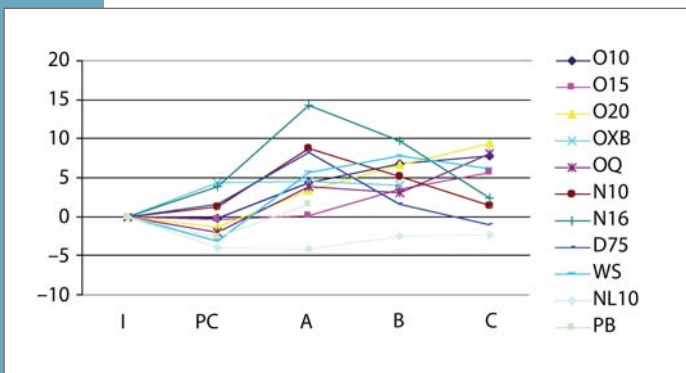


Fig 3c Differential b* dentin values following sample staining and subsequent applications of bleaching products (I = initial, PC = postcoloration, A = first 5 applications, B = total of 10 applications, and C = total of 20 applications; see Table 1 for product abbreviations).

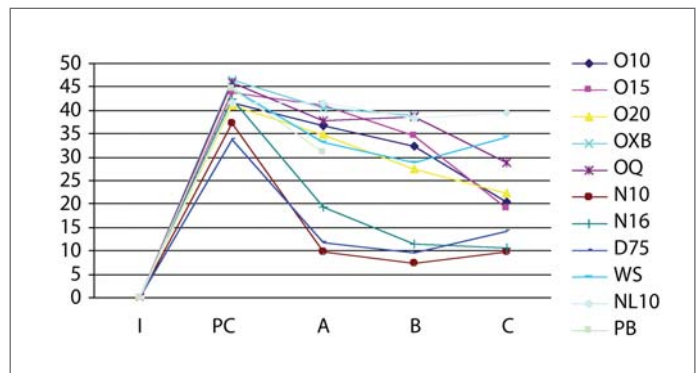


Fig 3d Global dentin color changes ΔE following sample staining and subsequent applications of bleaching products (I = initial, PC = postcoloration, A = first 5 applications, B = total of 10 applications, and C = total of 20 applications; see Table 1 for product abbreviations).

In contrast, all bleaching treatments produced an increase in L* values but of varying amplitude, especially on the dentin side (see Tables 2a and 2b and Figs 2a and 3a). As regards a* and b* values, some variations were also reported, usually as a reduction of the chroma (see Tables 2a and 2b and Figs 2b, 2c, 3b, and 3c).

Color differences following bleaching treatment (ΔE_2 to ΔE_4) were not significant between groups for enamel, while they were significant for dentin (see Table 3 and Figs 2d and 3d); actually, all treatments had a comparable lightening effect on enamel, while significant differences were reported as regards the potency of different bleaching products and protocols to reduce or suppress dentin dis-

coloration (Fig 4). Two products for home application (Nite White 10% and 16%, Day White 7.5%) showed a more pronounced bleaching effect in the tissue depth, as shown and measured on the dentin side, than in-office bleaching products (Opalescence Quick, Opalescence X-tra Boost, and Brite Smile), over-the-counter product (White Strips), and Nite White applied in a thin layer. Within the group of home bleaching products, Opalescence 10%, 15%, and 20% showed a less pronounced effect in tissue depth than Nite White 10% and 16% or Day White 7.5%, following the first 10 applications (ΔE_2 and ΔE_3). After 20 applications (ΔE_4), the difference was no longer significant.



Figs 4a and 4b Postbleaching samples showing the bleaching effect obtained on enamel (**a**) and dentin (**b**) following (from left to right) 20 applications of Nite White 10% (N10), 20 applications of Opalescence 10% (O10), and 5 applications of Brite Smile (PB). The limited in-depth bleaching effect achieved with the power bleaching approach (Brite Smile) is obvious.

Only Nite White (10% or 20%) and Day White allowed for a complete reversal of the dentin discoloration (after just 5 applications), while all other products showed only a progressive and incomplete pigment alteration, even after 20 applications (see Table 2b and Figs 2d and 3d). All products continued their bleaching effect on dentin after repeated application, irrespective of the final sample color improvement.

DISCUSSION

The staining technique applied to the samples of this study has been applied in several studies.^{27–29} This staining technique is based on the penetration of red blood cells and hemoglobin pigments inside the tooth structure from the dentin side of the samples. The rationale for staining the samples is to allow for a more discriminative comparison of the different bleaching methods and products and in particular the evaluation of their superficial (bleaching of enamel) or in-depth (bleaching of dentin) action. A preliminary evaluation also proved that it was practically impossible to reveal differences in the effect of various bleaching techniques on nonstained samples.

The absence of significant color difference between pre- and postcoloration values ($\Delta E1$) and almost complete absence of difference in $L^*a^*b^*$ measurements among the groups before and after coloration proves that a reasonably uniform sample quality and staining was achieved.

The use of bovine teeth for this study allowed the preparation of samples having standardized size and quality and also anatomically relevant tissue thickness. Likewise, it made possible the fabrication of samples whose dimensions are compatible with the size of the colorimeter measuring window. A last advantage of using this in vitro protocol with staining technique was the absence of clinical variables.

The color parameters were recorded in the $L^*a^*b^*$ color space, as established by the Commission Internationale de l'Eclairage (CIE) in 1976.^{32,33,35} The CIELab system is adequately related to human eye color perception in all 3 dimensions or directions of color space.³⁶ The Minolta CR-121 has been used in several dental research studies, including those for detection of color differences between metal-ceramic restorations, resin composite restorations, and also to evaluate the effects of bleaching.^{17,37–39}

ΔE depicts the global color change including the 3 dimensions of the CIE $L^*a^*b^*$ system and was therefore used to compare the efficacy of the different bleaching protocols; a ΔE below 1 is considered visually nondetectable, while values up to 3.3 are considered moderate visual differences.⁴⁰ When analyzing the 3 color dimensions separately, L^* values, which depict the sample lightness, appeared to be the most significant parameter, while a^* and b^* values, depicting chroma, proved less useful to make comparisons between products or experimental conditions.

In terms of a^* values, a general increase was measured for both dentin and enamel sides following staining; then, values dropped following treatment, proportionally to the efficacy of the bleaching product. A few significant differences were found regarding post-coloration a^* values, which are mostly related to the degree of hemoglobin oxidation; actually, a difference in the sample tint (red to brown) could be visualized. Slight variations were also observed in b^* values after blood staining. Posttreatment b^* enamel values tended to approach zero with effective bleaching products while no logical progression could be observed on the dentin side.

The main finding of the present study is the patent difference between in-office bleaching products (Opalescence Quick, Opalescence X-tra Boost, and Brite Smile), an over-the-counter brand (White Strips) or a modified home bleaching protocol (ultrathin gel layer simulating a bleaching tray without reservoir), and home bleaching systems, with prolonged application time. Actually, while a comparable bleaching effect on enamel was observed with all products and protocols under evaluation, the home bleaching technique only proved efficient in tissue depth. These findings confirm the observation of many clinicians.

As regards home bleaching, the potential of carbamide peroxide to penetrate enamel and bleach dentin rather uniformly was documented *in vitro*⁴¹ and *in vivo* in long-term clinical trials.^{11–13} In contrast, only scarce information is available concerning the efficacy and especially color stability achieved with in-office or so-called power bleaching techniques.^{16,31,42,43} The few studies attempting to evaluate the role of light activation generally fail to demonstrate a crucial effect^{15,44,45} or showed an improvement only for some bleaching materials.¹⁶ A reduced bleaching effect in tissue depth following chairside bleaching is rather logical as diffusion is essentially governed by a diffusion coefficient, time, and concentration of the bleaching active ingredients or ions¹⁰; the incorporation of chemical activators or the use of light or heat (produced by plasma, LED, or laser light units) as well as the use of higher concentrations of hydrogen peroxide could

actually not fully compensate for the reduced contact time between the bleaching product and tissues. It is therefore not surprising that several manufacturers propose some form of home-bleaching regimen to stabilize or reinforce the effect of chairside bleaching.⁴⁶ In consideration of the limited proof of efficacy of chairside bleaching, one can explain the rising interest of the dental industry and profession for this treatment modality^{42,47} by the reduced treatment time.

In comparison to Opalescence, a more rapid bleaching process in tissue depth was observed with Nite White and Day White, both of which contain similar concentrations of CP. In a clinical trial, however, no difference in the bleaching efficacy of both products was reported.⁴⁸ The only potential explanation for a more rapid bleaching effect of the Nite White products is the presence of H_2O_2 , which, combined with CP, equals a 10% or 15% pure CP gel. The faster penetration of H_2O_2 degradation products¹⁰ might then speed up the bleaching process, at least *in vitro*.

The use of higher concentrations of CP (15%, 16%, and 20%) did not show an increased bleaching effect on dentin with either Nite White or Opalescence products. In clinical studies, 15% CP showed a more significant color change at 2 weeks,^{49,50} while at 4 weeks posttreatment, no difference could be observed.⁵⁰ Likewise, 20% CP gel proved more efficient than 7.5% H_2O_2 at 2 weeks, the difference disappearing at 12 weeks.⁵¹

Another interesting finding is the limited in-depth bleaching effect of Nite White 10% when applied as a film rather than as a thick layer. The application of the bleaching gel as a thin layer was supposed to mimic a tray without a reservoir, which is a recent trend for the fabrication of bleaching trays.^{52,53} From the present results, it could be hypothesized that the quantity of active ingredient is insufficient to provide the same effect in tissue depth as with a thick bleaching gel layer. An *in vivo* evaluation of remaining CP within bleaching trays after 2 hours of treatment has actually shown larger quantities in reservoirs than in fitting trays, despite the fact that CP degraded at a similar rate in both types of trays.⁵⁴ On the other hand, in the present study conditions, one cannot exclude a par-

tial inactivation of the bleaching gel by either evaporation or drying on the sample surface (samples stored in a water-saturated environment but without direct protection). In real clinical conditions, however, thin trays might deform and therefore maintain a minimal space for the bleaching gel, thus creating more ideal conditions than the laboratory simulation of a perfectly fitting tray. A clinical report suggested that the absence of a reservoir does not reduce the bleaching effect at 10 days⁵⁵; this conclusion, however, was drawn following a short-term observation.

An additional reduction in dentin discoloration was also systematically correlated with an increasing number of bleaching product applications, irrespective of the final bleaching effect achieved. This observation suggests that, when prescribing a home bleaching treatment, clinicians should comply with the original protocol of about 20 applications of the bleaching agent; this should provide optimal effect and posttreatment color stability. Reports on the long-term effect of home bleaching have also confirmed the efficacy of the traditional protocol.^{11–13,56,57} Actually, the literature does not provide the same proofs for “revised” bleaching protocols, which usually promote a shorter application time and a reduced number of gel applications. It also needs to be pointed out that the large majority of clinical reports provide data only about the short-term effect of various bleaching products and protocols, while a follow-up over a 6- to 12-month period appears mandatory to evaluate the real treatment impact on tooth color and its stability.

The application of White Strips proved rather inefficient to bleach dentin, despite 20 applications under more ideal conditions than in vivo. In real conditions, strips are likely to move during function, swallowing, or phonation, and dissolution of the bleaching gel by saliva can occur, which potentially reduces the treatment efficacy even more. On the other hand, many studies have reported a satisfactory bleaching effect of the White Strips^{58,59} as well as a superior efficacy to other over-the-counter systems^{60–62} or an action equal to bleaching with custom trays and CP.^{58,60,63,64} However, in the later clinical trials, measurements were performed at the

end of the treatment, which does not take into consideration the rebound effect observed within the forthcoming days and weeks.⁶⁵ It has been shown that bleaching induces enamel and dentin demineralization, as measured by changes in microhardness values, which is followed by a remineralization process^{21–26}; this likely explains changes in tooth appearance such as the short-term higher enamel brightness and opacity. It therefore appears mandatory to assess the clinical efficacy of bleaching not only immediately after completion of the treatment but also after a few months at least, so that an objective comparison of treatment modalities can be performed.

CONCLUSION

An in vitro measurement of L*a*b* values of stained bovine teeth samples after bleaching with different products, concentrations, and protocols allowed for a discriminative comparison of the treatment efficacy on enamel surface and deeper dentin layers. The results of this trial allow the following conclusions:

- All treatment modalities under evaluation presented a similar bleaching effect on enamel but a markedly different action on dentin, with home bleaching being the most effective in tissue depth.
- The use of higher carbamide peroxide gels (15%, 16%, and 20% versus 10%) did not prove significantly more effective after 20 applications.
- The application of a thin layer of bleaching gel in vitro reduces the in-depth treatment effect.
- The self-directed White Strips proved less efficient than the home bleaching protocol.

Based on the results of this in vitro study in which in-office bleaching products demonstrated limited in-depth effect, further in vivo studies are required to evaluate whether in-office bleaching can be considered effective for initial light tooth shades or as initial treatment modality for darker shades that will have to be complemented by home bleaching.

REFERENCES

1. Fasanaro TS. Bleaching teeth: History, chemicals, and methods used for common tooth discolorations. *J Esthet Dent* 1992;4:70–78.
2. Black GV, McKay FS. Mottled enamel: An endemic developmental imperfection of the enamel of the teeth heretofore unknown in the literature of dentistry. *Dent Cosmos* 1916;58:129–156.
3. McKay FS. Mottled enamel. In: Black GV. *Pathology of the Hard Tissues of the Teeth: Oral Diagnosis*. Chicago: Medico-Dental, 1936:246–247.
4. Ames JW. Removing stains from mottled enamel. *J Am Dent Assoc* 1937;24:1674–1677.
5. McInnes J. Removing brown stains from teeth. *Ariz Dent J* 1966;12:13.
6. Schwachmann H, Schuster A. The tetracycline's applied pharmacology. *Pediatr Clin North Am* 1956;3:295–303.
7. Feinmann RA, Goldstein RE, Garber DA. *Bleaching Teeth*. Chicago: Quintessence, 1987:9–32.
8. Haywood VB, Heymann HO. Nightguard vital bleaching. *Quintessence Int* 1989;20:173–176.
9. Goldstein RE, Garber DA. *Complete Dental Bleaching*. Chicago: Quintessence, 1995.
10. Hanks CT, Fat JC, Wataha JC, Corcoran JF. Cytotoxicity and dentin permeability of carbamide peroxide and hydrogen vital bleaching materials, in vitro. *J Dent Res* 1993;72:931–938.
11. Leonard RH. Nightguard vital bleaching: Dark stains and long-term results. *Compend Contin Educ Dent* 2000;28:S18–27.
12. Leonard RH, Bentley C, Eagle JC, Garland GE, Knight MC, Phillips C. Nightguard vital bleaching: A long-term study on the efficacy, shade retention, side effects and patient's perceptions. *J Esthet Restor Dent* 2001;13:257–369.
13. Ritter AV, Leonard RH, St George AJ, Caplan DJ, Haywood VB. Safety and stability of nightguard vital bleaching: 9 to 12 years post-treatment. *J Esthet Restor Dent* 2002;14:275–285.
14. Li Y, Lee SS, Cartwright SL, Wilson AC. Comparison of clinical efficacy and safety of three professional at-home tooth whitening systems. *Compend Contin Educ Dent* 2003;24:357–360.
15. Papathanasiou A, Kastali S, Perry RD, Kugel G. Clinical evaluation of a 35% hydrogen peroxide in-office whitening system. *Compend Contin Educ Dent* 2002;23:335–338.
16. Luk K, Tam L, Hubert M. Effect of light energy on peroxide tooth bleaching. *J Am Dent Assoc* 2004;135:194–201.
17. Rosenstiel SF, Gegauff AG, Johnston WM. Duration of tooth color change after bleaching. *J Am Dent Assoc* 1991;122:54–59.
18. Bentley C, Leonard RH, Nelson CF, Bentley SA. Quantitation of vital bleaching by computer analysis of photographic images. *J Am Dent Assoc* 1999;130:809–816.
19. Rustogi KN, Curtis J. Development of a quantitative measurement to assess the whitening effects of two different oxygenating agents on teeth in vivo. *Compend Contin Educ Dent* 1994;17:S631–S634.
20. Amaechi BT, Higham SM. Development of a quantitative method to monitor the effect of a tooth whitening agent. *J Clin Dent* 2002;13:100–103.
21. Basting RT, Rodrigues AL Jr, Serra MC. The effect of 10% carbamide peroxide bleaching material on microhardness of sound and demineralized enamel. *Oper dent* 2001;26:531–539.
22. Basting RT, Rodrigues AL Jr, Serra MC. The effect of seven carbamide peroxide bleaching agents on enamel microhardness over time. *J Am Dent Assoc* 2003;134:1335–1342.
23. De Freitas PM, Turssi CP, Hara AT, Serra MC. Monitoring of dentin microhardness throughout and after bleaching. *Am J Dent* 2004;17:342–346.
24. De Freitas PM, Turssi CP, Hara AT, Serra MC. Dentin microhardness during and after bleaching. *Quintessence Int* 2004;35:411–417.
25. Cimilli H, Pameijer CH. Effect of carbamide peroxide bleaching agents on the physical properties and chemical composition of enamel. *Am J Dent* 2001;14:63–66.
26. Potocknic I, Kosec L, Gaspersic D. Effect of 10% carbamide peroxide bleaching gel on enamel microhardness, microstructure and mineral content. *J Endod* 2000;26:203–206.
27. Freccia WF, Peters DD. A technique for staining extracted teeth: A research and teaching aid for bleaching. *J Endod* 1982;8:67–69.
28. Rotstein I, Zalkind M, Mor C, Tarabeah A, Friedman S. In vitro efficacy of sodium perborate preparations used for intracoronal bleaching of discolored non-vital teeth. *Endod Dent Traumatol* 1991;7:177–180.
29. Vachon C, Vanek P, Friedman S. Internal bleaching with 10% carbamide peroxide in vitro. *Pract Periodontics Aesthet Dent* 1998;10:1145–1154.
30. Sulieman M, Addy M, Rees JS. Development and evaluation of a method in vitro to study the effectiveness of tooth bleaching. *J Dent* 2003;31:415–422.
31. Sulieman M, Addy M, McDonald E, Rees JS. The bleaching depth of a 35% peroxide-based in-office product: A study in vitro. *J Dent* 2005;33:33–40.
32. Commission Internationale de l'Eclairage. *Colorimetry*. Publication No. 15 1976, supplement No. 15.
33. Chamberlain GJ, Chamberlain DG. *Colour: Its measurement, computation and application*. London: Heyden & Son, 1980:60–61.
34. Sachs L. *Angewandte Statistik: Planung und Auswertung, Methoden und Modelle*. Berlin: Springer, 1974:420–429.

35. Clarke FJ. Measurement of colour of human teeth. In: McLean JW. *Dental Ceramic: Proceedings of the First International Symposium on Ceramics [23–25 Apr 1982, New Orleans]*. Chicago: Quintessence, 1983:441–489.
36. Kuehni RG. Color-tolerance data and the tentative CIE 1976 Lab formula. *J Opt Soc Am* 1976;66: 497–500.
37. Rosenstiel SF, Porter SS, Johnston WM. Color measurements of all ceramic crown systems. *J Prosthet Dent* 1988;60:297–303.
38. Johnston WM, Kao EC. Assessment of appearance match by visual observation and clinical colorimetry. *J Dent Res* 1989;68:819–822.
39. Dietschi D, Campanile G, Holz J, Meyer JM. Comparison of the color stability of ten new-generation composites: An in vitro study. *Dent Mater* 1994;10:353–362.
40. Um CM, Ruyter IE. Staining of resin-based veneering materials with coffee and tea. *Quintessence Int* 1991;22:377–386.
41. McCaslin AJ, Haywood VB, Potter BJ, Dickinson GL, Russel CM. Assessing dentin color changes from nightguard vital bleaching. *J Am Dent Assoc* 1999;130:1485–1490.
42. Miller MB. Power bleaching: Does it work or is it marketing hype? *Pract Proced Aesthetic Dent* 2002;14:636.
43. Yurdukuru B, Akoren AC, Unsal MK. Alterations in human enamel surface morphology following the use of an office bleaching agent and consecutive application of 37% phosphoric acid in vivo. *J Clin Dent* 2003;14:103–107.
44. Jones AH, Diaz-Arnold AM, Vargas MA, Cobb DS. Colorimetric assessment of laser and home bleaching techniques. *J Esthet Dent* 1999;11:87–94.
45. Dostalova T, Jelinkova H, Housova D, et al. Diode laser-activated bleaching. *Braz Dent J* 2004;15: S13–18.
46. Kugel G, Perry RD, Hoang E, Scherer W. Effective tooth bleaching in 5 days: Using a combined in-office and at-home bleaching system. *Compend Contin Educ Dent* 1997;18:378–383.
47. Miller MB. Power bleaching attempts to make a comeback. *Dent Today* 1992;11:35–37.
48. Cibirka RM, Myers M, Downey MC, et al. Clinical study of tooth shade lightening from dentist-supervised, patient-applied treatment with two 10% carbamide peroxide gels. *J Esthet Dent* 1999;11:325–331.
49. Kihn PW, Barnes DM, Romberg E, Peterson K. A clinical evaluation of 10 percent vs. 15 percent carbamide peroxide tooth-whitening agents. *J Am Dent Assoc* 2000;131:1478–1484.
50. Matis BA, Mousa HN, Cochran MA, Eckert GJ. Clinical evaluation of bleaching agents of different concentrations. *Quintessence Int* 2000;31:303–310.
51. Mokhlis GR, Matis BA, Cochran MA, Eckert GJ. A clinical evaluation of carbamide peroxide and hydrogen peroxide whitening agents during daytime use. *J Am Dent Assoc* 2000;131:1269–1277.
52. Miller MB, Castellanos IR, Rieger MS. Efficacy of home bleaching with and without tray reservoirs. *Pract Periodontics Aesthet Dent* 2000;12:611–614.
53. Matis BA, Yousef M, Cochran MA, Eckert GJ. Degradation of bleaching gels in vivo as a function of tray design and carbamide peroxide concentration. *Oper Dent* 2002;27:12–18.
54. Matis BA, Hamdan YS, Cochran MA, Eckert GJ. A clinical evaluation of a bleaching agent used with and without reservoirs. *Oper Dent* 2002;27:5–11.
55. Javaheri DS, Janis JN. The efficacy of reservoirs in bleaching trays. *Oper Dent* 2000;25:149–151.
56. Dunn JR. Dentist-prescribed home bleaching: Current status. *Compend Contin Educ Dent* 1998;19:760–764.
57. Swift EJ Jr, May KN, Wilder AD Jr, Heymann HO, Bayne SC. Two-year clinical evaluation of tooth whitening using an at-home bleaching system. *J Esthet Dent* 1999;11:36–42.
58. Sagel PA, Odioso LL, McMillan DA, Gerlach RW. Vital tooth whitening with a novel hydrogen peroxide strip system: Design, kinetics, and clinical response. *Compend Contin Educ Dent* 2000;29:S10–S15.
59. Gerlach RW, Zhou X. Vital bleaching with whitening strips: Summary of clinical research on effectiveness and tolerability. *J Contemp Dent Pract* 2001;15: 1–16.
60. Gerlach RW, Zhou X. Comparative clinical efficacy of two professional bleaching systems. *Compend Contin Educ Dent* 2002;23:35–41.
61. Gerlach RW, Barker ML. Randomized clinical trial comparing overnight use of two self-directed peroxide tooth whiteners. *Am J Dent* 2003;16(special issue):17B–21B.
62. Karpinia KA, Magnusson I, Barker ML, Gerlach RW. Comparison of two self-directed bleaching systems. *J Prosthodont* 2003;12:242–248.
63. Gerlach RW, Gibb RD, Sagel PA. A randomized clinical trial comparing a novel 5.3% hydrogen peroxide whitening strip to 10%, 15%, and 20% carbamide peroxide tray-based bleaching systems. *Compend Contin Educ Dent* 2000;29:S22–S28.
64. Karpinia KA, Magnusson I, Sagel PA, Zhou X, Gerlach RW. Vital bleaching with two at-home professional systems. *Am J Dent* 2002;15(special issue):13A–18A.
65. Deliperi S, Bardwell DN, Papathanasiou A. Clinical evaluation of a combined in-office and take-home bleaching system. *J Am Dent Assoc* 2004;135: 628–634.